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Local Lipid Reorganization by a Transmembrane Protein Domain

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Abstract

Membrane proteins interact with their lipid bilayer environment via both a transmembrane helix and juxtamembrane regions. The effect of juxtamembrane regions and membrane lipid composition on these interactions has been explored by multi-scale molecular dynamics simulations. The consequences of anionic lipids within the inner leaflet of a membrane were studied in combination with membrane spanning protein models differing in their juxtamembrane domains. The simulations reveal sensitivity of the protein-lipid interactions to membrane lipid composition and charged amino acid sidechains. Basic residues on the intracellular side of the protein facilitated interactions with anionic lipids. Protein systems without basic residues do not show selectivity for anionic compared to zwitterionic lipids. This reveals the sensitivity to both the composition of the membrane and the protein system when studying membrane embedded proteins. The results presented here illustrate how even simple transmembrane domain is able to induce lipid reorganization in a mixed asymmetric bilayer.

abstract graphic

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Supporting Information Available: Computational details of the simulations and their analysis. This material is available free of charge via the Internet at <http://pubs.acs.org>.

Keywords

Coarse-grained simulations; membrane composition; lipid asymmetry

It has been estimated that ca. 25% of all open reading frames in any genome encode α -helical membrane proteins¹. We therefore need to characterize fully the interactions of transmembrane (TM) α -helices with the membrane lipids, to understand the biophysical chemistry of membrane protein stability and to aid membrane protein structure prediction².

TM α -helices contain a core of hydrophobic amino acids in order to stably span the hydrophobic core of the bilayer³. Amphipathic aromatic (Trp, Tyr) residues and basic (Arg, Lys) residues are localized preferentially at the ends of TM α -helices, where they interact respectively with acyl and phosphate groups of phospholipids⁴. Also, positively charged residues in intracellular loops between TM helices help determine the topology of membrane proteins⁵, possibly via their interactions with negatively charged phospholipids⁶.

The organization of cell membranes depends on the interplay between lipids and TM α -helices, which can be explored directly via molecular simulations, using either all atom molecular dynamics (AT-MD)^{7, 8}, coarse-grained molecular dynamics (CG-MD)^{9, 10}, or dissipative particle dynamics (DPD)¹¹. Such simulations allow detailed exploration of interactions between hydrophobic TM α -helices and lipids, of the effects of TM helices on lipid phase behavior¹², and of effects of helix/bilayer length mismatch on lipid distribution around membrane proteins^{8, 13}. Both the latter effects may contribute to formation of nanoscopic lipid clusters/domains within complex cell membranes, a topic of biological importance¹⁴.

Cytokine and related receptors¹⁵ provide a model system for studying bilayer interactions of the TM domains of membrane proteins. These receptors have a large extracellular domain followed by a single hydrophobic TM helix and a cytoplasmic tail. Immediately following the TM helix is a flexible juxtamembrane (JM) domain. JM domains of both cytokine receptors and of receptor tyrosine kinases are rich in basic amino acids and may interact with anionic lipids in the inner leaflet of the bilayer¹⁶. The TM+JM system of such receptors thus provide test systems to explore aspects of protein/lipid interactions additional to hydrophobic interactions between TM helix and the bilayer core. Interactions of the JM region with complex lipid bilayers have to date not been extensively explored.

We have used the TM and JM domains of the gp130 receptor protein^{15, 17} as a model system to examine the influence of the JM domain on the local bilayer composition. Three models were used to explore the effect of charged residues on interactions with lipids. **TM** contained just the 22 predominantly hydrophobic amino acids of the predicted TM α -helix. The other models contained four (**TM+4**) or eight (**TM+8**) additional residues at each end of the TM helix, as flexible coils (Fig. 1). Each model was embedded in a zwitterionic (POPC) lipid bilayer via self-assembly CG-MD simulations¹⁸ (see Supporting Information for details). Three simulations of each system were run for 1 μ s of CG-MD simulation using different random seeds. At the end of these simulations, 10 % of the POPC lipids (inner

leaflet) were exchanged to anionic (POPS) lipids (Fig. 1C) to mimic the intracellular lipids of mammalian cell membranes¹⁹. The resultant asymmetric bilayer systems were each simulated for 1 μ s of CG-MD. Comparison of root mean square fluctuations for the backbones of the CG and AT simulations

To analyze the effect of protein on local ordering of the lipids, spherical radial distribution functions (RDFs) of the lipids with respect to the protein were calculated (Fig. 2). RDFs of the head groups and the tails of both POPC and POPS were calculated. In the asymmetric bilayer simulation the POPC lipid tails show similar behavior in all three systems (**TM**, **TM+4** and **TM+8**; Fig. 2A-C). However, the head group phosphate particles of POPC in the **TM** system do show a slightly higher probability of occurrence within the first interaction shell (~ 5 Å). In contrast, there are large differences between the three different protein systems when comparing the RDFs of POPS (Fig. 2D-F). In particular there is a significant increase in the probability of POPS lipids within the first interaction shell when basic residues are present in the JM regions of the protein model (i.e. in **TM+4** and **TM+8**). This indicates favorable electrostatic interaction between the positively charged JM regions of the protein and the anionic lipid head groups.

In addition to the system specific behavior of the lipids, the RDFs of the lipid tails show a distinct pattern in all three systems. For both lipid types, the highest probability distance is observed around 5 Å corresponding to the first interaction shell. The RDFs contain several successive peaks with decreasing probability at 10, 15 and 20 Å, indicating that the lipids are organized in ordered ring-like patterns around the protein. Thus even a relatively simple TM α -helix monomer has an impact on the dynamics and ordering of the surrounding lipids. Comparable interactions of TM proteins and lipids have been seen in more complex systems²⁰.

Ordering of lipids can be explored by the visualization of density maps of the spatial occupancy of lipid head groups around the protein during the simulations (Fig. 3). These maps show local clustering of the anionic lipids around positively charged residues of the protein in the **TM+4** and **TM+8** systems, while no such clustering is observed in the **TM** system. This suggests that positively charged groups of the JM region control the selection of lipid types in proximity to the protein.

We analyzed the effects of protein model and lipid composition on lateral diffusion of lipids within the membranes. (To improve statistics on diffusion of lipids, the asymmetric bilayer simulations were extended to 2 μ s.) One might anticipate that interactions between positively charged residues and anionic lipids would influence diffusion rates of the lipids. There is a small effect of the JM regions on the diffusion of the anionic (POPS) lipids. For the **TM** simulations the diffusion coefficient for the POPS lipids is $9.5 \times 10^{-7} \text{ cm}^2 \text{ s}^{-1}$. This coefficient decreases in the **TM+4** and **TM+8** simulations to $6.7 \times 10^{-7} \text{ cm}^2 \text{ s}^{-1}$ and $6.8 \times 10^{-7} \text{ cm}^2 \text{ s}^{-1}$ respectively.

The 1 μ s snapshots of each of the CG simulations in POPC/POPS were used to initiate 50 ns atomistic MD simulations. The dynamics of the protein in the CG and AT simulations were compared utilizing root mean square fluctuation calculations (Fig S2A). It is evident that the

overall dynamical behaviour of the protein is similar in the CG and AT simulations, which indicates that the elastic network applied in the CG simulations does not adversely impact the dynamics of the flexible JM regions of the protein. This is further illustrated by the overlay of simulations snapshots from both the **TM+8** PC+PS CG simulations and the **TM+8** atomistic simulations, which indicates that the JM regions are highly mobile in both in the CG and the AT simulations (Fig. S2B-C). Also the displacement of the α -helix with respect to membrane does not seem to differ between the CG and AT simulations. As with the corresponding CG simulations, density maps of the spatial occupancy of lipid head groups around the protein (Fig. 4) reveal local clustering of POPS around the positively charged Arg and Lys sidechains of the JM regions of **TM+4** and **TM+8**.

The interactions patterns between Arg and Lys and the POPS head groups in the atomistic simulations reveal that the basic sidechains interact with both of the anionic moieties (i.e. the carboxylate and the phosphate). The increased attraction of the anionic lipids compared to the zwitterionic lipids towards the basic residues therefore seems to be an effect of favorable charge interactions in addition to decreased charge repulsion observed which could occur with the cationic choline group of POPC.

Our results reveal the sensitivity of protein-lipid interactions to the nature of the lipids and of the transmembrane protein. We observe a distinct ordering of the phospholipids around the protein within the first interaction shell around 5 Å, with subsequent peaks also observed at 10 Å, 15 Å and 20 Å with decreasing probability. This ordering of lipids around the protein is observed both for the zwitterionic and the anionic lipids. However, the presence of positively charged JM residues leads to local clustering of anionic lipids within these shells. Thus it is clear that even a simple (i.e. single TM helix plus short JM region) transmembrane domain is able to induce lipid nanodomain formation around a protein. Furthermore proteins containing basic residues in their JM regions are able to selectively form specific interactions with the anionic lipids. This agrees with the *in vivo* composition of cell membranes²¹ where anionic lipids are located in the intracellular side of the cell¹⁹, and with the “positive inside rule”²² which suggests that the topology of integral membrane proteins is dictated by the location of positively charged residues adjacent to the hydrophobic TM helix. Interestingly, comparable effects on lipid reorganization have also been seen for membrane-binding peptides e.g. from viral fusion proteins²³.

In summary, it is clear that even for simple systems a positively charged JM region can reorganize the local bilayer environment. This is of importance both in larger scale organization of lipid nanoclusters in membranes²⁴, and also because it may modulate helix/helix dimerization within lipid bilayers, a key event in cytokine and related receptor signalling and in membrane protein folding.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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References

- (1). Wallin E, von Heijne G. Genome-Wide Analysis of Integral Membrane Proteins from Eubacterial, Archean, and Eukaryotic Organisms. *Prot. Sci.* 1998; 7:1029–1038.
- (2). Nugent T, Jones DT. Accurate De Novo Structure Prediction of Large Transmembrane Protein Domains Using Fragment-Assembly and Correlated Mutation Analysis. *Proc. Natl. Acad. Sci. USA.* 2012; 109:E1540–E1547. [PubMed: 22645369]
- (3). von Heijne G. Membrane Proteins: From Sequence to Structure. *Ann. Rev. Biophys. Biomol. Struct.* 1994; 23:167–192. [PubMed: 7919780]
- (4). Killian JA, von Heijne G. How Proteins Adapt to a Membrane-Water Interface. *Trends Biochem. Sci.* 2000; 25:429–434. [PubMed: 10973056]
- (5). Seppala S, Slusky JS, Lloris-Garcera P, Rapp M, von Heijne G. Control of Membrane Protein Topology by a Single C-Terminal Residue. *Science.* 2010; 328:1698–1700. [PubMed: 20508091]
- (6). van Klompenburg W, Nilsson I, von Heijne G, de Kruijff B. Anionic Phospholipids Are Determinants of Membrane Protein Topology. *EMBO J.* 1997; 16:4261–4266. [PubMed: 9250669]
- (7). Woolf TB. Molecular Dynamics of Individual α -Helices of Bacteriorhodopsin in Dimyristoyl Phosphatidylcholine. I. Structure and Dynamics. *Biophys. J.* 1997; 73:2376–2392. [PubMed: 9370432]
- (8). Kandasamy SK, Larson RG. Molecular Dynamics Simulations of Model Trans-Membrane Peptides in Lipid Bilayers: A Systematic Investigation of Hydrophobic Mismatch. *Biophys. J.* 2006; 90:2326–2343. [PubMed: 16428278]
- (9). Bond PJ, Wee CL, Sansom MSP. Coarse-Grained Molecular Dynamics Simulations of the Energetics of Helix Insertion into a Lipid Bilayer. *Biochem.* 2008; 47:11321–11331. [PubMed: 18831536]
- (10). Monticelli L, Kandasamy SK, Periole X, Larson RG, Tieleman DP, Marrink SJ. The Martini Coarse Grained Force Field: Extension to Proteins. *J. Chem. Theor. Comp.* 2008; 4:819–834.
- (11). de Meyer FJM, Venturoli M, Smit B. Molecular Simulations of Lipid-Mediated Protein-Protein Interactions. *Biophys. J.* 2008; 95:1851–1865. [PubMed: 18487292]
- (12). Domanski J, Marrink SJ, Schäfer LV. Transmembrane Helices Can Induce Domain Formation in Crowded Model Membranes. *Biochim. Biophys. Acta.* 2012
- (13). Schäfer LV, de Jong DH, Holt A, Rzeplia AJ, de Vries AH, Poolman B, Killian JA, Marrink SJ. Lipid Packing Drives the Segregation of Transmembrane Helices into Disordered Lipid Domains in Model Membranes. *Proc. Natl. Acad. Sci. USA.* 2011; 108:1343–1348. [PubMed: 21205902]
- (14). Spira F, Mueller NS, Beck G, von Olshausen P, Beig J, Wedlich-Söldner R. Patchwork Organization of the Yeast Plasma Membrane into Numerous Coexisting Domains. *Nature Cell Biol.* 2012; 14:640–648. [PubMed: 22544065]
- (15). Broughton SE, Hercus TR, Lopez AF, Parker MW. Cytokine Receptor Activation at the Cell Surface. *Curr. Opin. Struct. Biol.* 2012; 22:350–359. [PubMed: 22521507]
- (16). Michailidis IE, Rusinova R, Georgakopoulos A, Chen Y, Iyengar R, Robakis NK, Logothetis DE, Baki L. Phosphatidylinositol-4,5-Bisphosphate Regulates Epidermal Growth Factor Receptor Activation. *Pflugers Arch.* 2011; 461:387–397. [PubMed: 21107857]
- (17). Lupardus PJ, Skiniotis G, Rice AJ, Thomas C, Fischer S, Walz T, Garcia KC. Structural Snapshots of Full-Length Jak1, a Transmembrane Gp130/IL-6/IL-6 α Cytokine Receptor Complex, and the Receptor-Jak1 Holocomplex. *Structure.* 2011; 19:45–55. [PubMed: 21220115]
- (18). Scott KA, Bond PJ, Ivetac A, Chetwynd AP, Khalid S, Sansom MSP. Coarse-Grained Molecular Dynamics Simulations of Membrane Protein-Bilayer Self-Assembly. *Structure.* 2008; 16:621–630. [PubMed: 18400182]

- (19). van Meer G, de Kroon AIPM. Lipid Map of the Mammalian Cell. *J. Cell Sci.* 2011; 124:5–8. [PubMed: 21172818]
- (20). Niemelä PS, Miettinen MS, Monticelli L, Hammaren H, Bjelkmar P, Murtola T, Lindahl E, Vattulainen I. Membrane Proteins Diffuse as Dynamic Complexes with Lipids. *J. Am. Chem. Soc.* 2010; 132:7574–7575. [PubMed: 20469857]
- (21). Shevchenko A, Simons K. Lipidomics: Coming to Grips with Lipid Diversity. *Nature Rev. Molec. Cell Biol.* 2010; 11:593–598. [PubMed: 20606693]
- (22). von Heijne G, Gavel Y. Topogenic Signals in Integral Membrane-Proteins. *Eur. J. Biochem.* 1988; 174:671–678. [PubMed: 3134198]
- (23). Vitiello G, Falanga A, Galdiero M, Marsh D, Galdiero S, D'Errico G. Lipid Composition Modulates the Interaction of Peptides Deriving from Herpes Simplex Virus Type I Glycoproteins B and H with Biomembranes. *Biochim. Biophys. Acta Biomembranes.* 2011; 1808:2517–2526.
- (24). van den Bogaart G, Meyenberg K, Risselada HJ, Amin H, Willig KI, Hubrich BE, Dier M, Hell SW, Grubmüller H, Diederichsen U, et al. Membrane Protein Sequestering by Ionic Protein-Lipid Interactions. *Nature.* 2011; 479:552–555. [PubMed: 22020284]

Figure 1.

Simulation systems. **A** Sequence of the transmembrane domain of gp130 and the immediately juxtamembrane regions. Basic residues are highlighted in blue, and acidic residues in red. The three horizontal lines indicate the extents of the core transmembrane domain (**TM**) and of the extended (**TM+4** and **TM+8**) sequences which include the juxtamembrane regions. **B** Model of **TM+8** with the central TM domain (from A620 to F641) modeled as an α -helix, while the juxtamembrane regions are modeled as random coils. **C** An initial CG simulation setup with **TM+8** embedded in a asymmetric bilayer in which 10 % of the (inner leaflet) POPC lipids have been exchanged for POPS. Water and ions have been omitted for clarity.

Figure 2.

Radial distribution function of lipid head groups (blue lines) and fatty acyl tails (green lines) with respect to the protein for all three CG simulation repeats for all 3 models in the POPC/POPS simulations. Since the proteins differ in length only the center part of the protein identical in all three systems was used in the calculations. **A-C** Radial distribution functions of POPC in the **TM**, **TM+4** and **TM+8** POPC/PS simulations respectively. **D-F** Radial distribution functions of POPS in the **TM**, **TM+4** and **TM+8** POPC/PS simulations respectively.

Figure 3.

Occupancy density plots of the CG lipid head group phosphate particles for POPC (blue) and for POPS (green). The occupancy density for the positively charged sidechain (Arg and Lys) of the membrane protein models is shown in magenta. The left-hand images show the side views of the **TM**, **TM+4** and **TM+8** density surfaces respectively, whereas the right-hand images show corresponding images viewed facing the inner leaflet of the bilayer.

Figure 4.

Occupancy plots for atomistic (AT-MD) simulations of **TM**, **TM+4** and **TM+8** in POPC/POPS. The view is facing the inner leaflet of the bilayer. Occupancy density plots of the phosphorus atom of the POPC head groups are shown in blue, of the phosphorus atoms of the POPS head group in green, and of the N η 1, N η 2 and N ϵ atoms of Arg and N ζ atoms of Lys sidechains are shown in magenta.